# Correspondence

AIDS 1991, 5:597–609

**Detection of HIV-1 RNA in factor VIII concentrate**

We wish to report the identification of HIV-1 RNA in an archival batch of native commercial factor VIII. Seventeen samples of factor VIII and one sample of factor IX concentrate were studied using a modified version of a new method for the rescue of cell-free viral RNA [1]. Samples came from individual batches, each of which had a different method of purification, date of production and selection of plasma donors (Table 1).

RNA was extracted by incubation of reconstituted blood-product concentrate in a buffer containing proteinase-K and HIV-1 cDNA synthesised as previously described using two different strategies. In one, random hexamers, and in the other HIV-1-specific primers (MH6), were used as primers for reverse transcription. All samples were randomized prior to both procedures which were conducted blind.

HIV-1-specific cDNA was detected by a nested polymerase chain reaction (PCR) using POL primers derived from the HIV-1 HXB2 sequence [2]. In the first round the outer primers were MH5 (sense) 5'GCAGGGGCAAGGCCAATGGACAT and MH6 (antisense) 5'CTCCCACTCAGGAATCCAGGTGGC and in the second round the inner primers were POL1 (sense) 5'CAGGAAAATATGCAAGAATGAGG and POL2 (anti-sense) 5'CCCATGTTTCCTTTTGTATGGGT. To control for proviral DNA, the RNA extract was also subjected to PCR without prior reverse transcription.

The reactions were carried out in a 50 µl volume containing 10 mmol/l Tris-HCl pH 8.3, 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 0.01% gelatin (w/v), 1.25 units Taq polymerase ('Amplitaq' Perkin Elmer Cetus), 200 µmol/l of each dNTP and 0.1 µmol/l of each primer. Approximately 25 µl of sterile mineral oil was layered above the solution to prevent evaporation.

In the first round of PCR 10 µl of factor VIII-derived cDNA was amplified (equivalent to 5 µl of reconstituted factor VIII concentrate) with outer primers MH5 and MH6, in a total of 50 µl of the reaction mix. Thermal cycling for the first round consisted of 1 cycle at 94°C for 4 min, 35 cycles at 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and finally, 1 cycle at 72°C for 7 min. (HYBAID programmable thermal cycler.) In the second round of PCR, 2 µl of each product of the first round were amplified with inner primers POL1 and POL2 in 50 µl reaction mix. Thermal cycling consisted of 25 cycles at 90°C for 1 min, 50°C for 1 min, 72°C for 1 min, and finally, 1 cycle at 72°C for 7 min.

**Table 1.** HIV-1 RNA sequences in clotting factor concentrates

| Sample | Expiry date | Donors† | Virucidal treatment | PCR results | |
|---|---|---|---|---|---|
| | | | | HIV (Rn) | HIV (Sp) |
| 1 VIII | June 1979 | Paid* | Unheated | − | − |
| 2 VIII | May 1981 | Paid* | Unheated | − | − |
| 3 VIII | Jan 1982 | Paid* | Unheated | − | − |
| 4 VIII | Apr 1983 | Paid* | Unheated | + | + |
| 5 VIII | Apr 1983 | Paid* | Unheated | − | − |
| 6 VIII | Nov 1984 | Paid* | Unheated | − | − |
| 7 VIII | Sep 1984 | Paid* | Dry heat, 60°C/32 h | − | − |
| 8 VIII | Jul 1985 | Paid* | Dry heat, 60°C/32 h | − | − |
| 9 VIII | Dec 1986 | Paid* | Heptane, 60°C/20 h | − | − |
| 10 VIII | Jun 1988 | Paid* | Heptane, 60°C/20 h | − | − |
| 11 VIII | Dec 1986 | Paid | Heptane, 60°C/20 h | − | − |
| 12 VIII | Apr 1989 | Paid | Heptane, 60°C/20 h | − | − |
| 13 VIII | Jul 1989 | Paid | Solvent/detergent | − | − |
| 14 VIII | Sep 1981 | Volunteer | Unheated | − | − |
| 15 VIII | Jul 1984 | Volunteer* | Unheated | − | − |
| 16 VIII | Jan 1986 | Volunteer | Dry heat, 80°C/72 h | − | − |
| 17 VIII | Sep 1989 | Volunteer | Dry heat, 80°C/72 h | − | − |
| 18 IX | Mar 1990 | Volunteer | Dry heat, 80°C/72 h | − | − |

Rn, Random priming; Sp, specific priming; +, HIV-1 RNA detected; −, HIV-1 RNA not detected; *, HCV RNA detected [5]; †, Paid, USA or European commercial donors; volunteer, UK donors.

Twenty microlitres of each product solution were run by submarine horizontal gel electrophoresis in a 2% agarose gel stained with ethidium bromide for 1 h at a potential of 100 volts and visualized by ultraviolet transillumination.

We demonstrated HIV-1 RNA in one of 18 samples of clotting factor concentrate only. It was detected by both methods of cDNA synthesis and no PCR signal was found in the absence of reverse transcription.

Titration of the cDNA indicated a minimum of $10^2$ viral copies per ml. Allowing for no more than 5% efficiency of reverse transcription [3], it is likely that this product contained at least $2 \times 10^3$ viral copies per ml.

The HIV-1-contaminated concentrate was released for use at the end of 1982. The total batch at the Sheffield centre was given to two haemophiliacs during emergency treatment. Both patients are now known to be anti-HIV-1-antibody positive; information on exact dates of seroconversion is incomplete but is known to post-date their documented treatment times.

From this small study in which one out of 18 samples contained HIV-1 RNA, it is not possible to determine the overall prevalence of HIV-1 in commercial blood concentrates although an association between patient seroconversion for anti-HIV-1 and exposure to commercial concentrates has been shown [4].

It is interesting that the single sample containing HIV-1 RNA would have been collected from commercial donors prior to the introduction of AIDS exclusion criteria and the manufacture would not have included a viral inactivation step. In practice, only the six samples 2 to 6 and 15 (Table 1) would be considered likely to contain HIV-1. This being so, our rate of detection of HIV-1 RNA in native concentrates produced after the establishment of HIV-1 infection in the donor panel is likely to be nearer to one in six.

We feel that the method described may be valuable in the study of archival concentrates and may have a use in the exclusion of HIV-1 from products made from pooled blood. Further work is now in progress using similar methods to detect and quantify cell-free HIV-1 in the sera and plasma of patients with HIV-1 infection.

## Acknowledgements

We thank Dr Jeremy Garson, Philip Tuke and Christopher Ring for their help.

***M.G. Semple, C. Loveday, E. Preston* and R.S. Tedder,*** *Division of Virology, Medical Microbiology, University College and Middlesex School of Medicine, London W1P 7PN and the *Department of Haematology, Royal Hallamshire Hospital, Sheffield, UK.*

*Date of receipt: 19 December 1990.*

## References

1. GARSON JA, TEDDER RS, BRIGGS M, *ET AL.*: **Detection of hepatitis C viral sequences in blood donation by 'nested' polymerase chain reaction and prediction of infectivity.** *Lancet* 1990, 335:1419–1422.
2. RATNER L, FISHER A, JAGODZINISKI LL, *ET AL.*: **Complete nucleotide sequences of functional clones of the AIDS virus.** *AIDS Res Hum Retroviruses* 1987, 3:57–69.
3. SIMMONDS P, ZHANG LQ, WATSON HG, *ET AL.*: **Hepatitis C quantification and sequencing in blood products, haemophiliacs and drug abusers.** *Lancet* 1990, 336:1469–1472.
4. CHEINGSONG-POPOV R, TEDDER RS, O'CONNOR T, *ET AL.*: **Retroviral infections among patients treated in Britain with various clotting factors.** *BMJ* 1985, 293:168–169.
5. GARSON JA, PRESTON FE, MAKRIS M, *ET AL.*: **Detection by PCR of hepatitis C virus in factor VIII concentrate.** *Lancet* 1990, 335:1473.

**Saliva can contribute in quick inhibition of HIV infectivity**

An anti-HIV factor was once described in the saliva of healthy adults and children. However, full inhibition in saliva alone is not achieved until the virus has been incubated for 1 h [1,2]. According to Fultz [1], this potential does not depend upon salivary peroxidase. It could, however, be associated with a specific macromolecule or molecular aggregate as suggested by Fox *et al.* [2]. Nevertheless, we found evidence that another efficient and much quicker agent could be generated by salivary peroxidase.

Permanent ARV-4 cell line, which chronically produces HIV, was used as a source for the virus in these experiments. Aliquots from the clarified supernatants were first tested against pre-incubation mixtures containing or not the following ingredients: (1) saliva, filtrated through 0.45 µ porosity filter and (2) glucose + glucose–oxidase (G-GO), an $H_2O_2$-producing medium. G and GO were adjusted at the ratio of 1000 µg:1 µg. GO final concentrations were 4 µg/ml; 1 µg/ml or 0.2 µg/ml: A HEPES buffer helped to keep